PROTOCOL FOR MOUSE DOSIMETRY EXPERIMENTS ON WALTON HORIZONTAL SMOKING MACHINE

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This protocol outlines proposed collaborative studies by Microbiological Associates and Oak Ridge National Laboratory to investigate mouse dosimetry on the Walton Horizontal Smoking Machine. The objectives of these studies are:

(1) To define the dose of tobacco smoke particulates received by mice under selected exposure conditions (exposure time and smoke concentration),

(2) To ascertain possible effects of sex and strain on dosimetry so that a rational selection can be made for future studies of biological impact,

(3) To determine retention and clearance rate of smoke particulates after exposure, and

(4) To determine cumulative dose and long-term retention of smoke particulates under typical exposure regimes.

Responsibilities of Oak Ridge National Laboratory are the following:

(1) Weight and RTD selection of cigarettes.
(2) Labeling of cigarettes with ^{14}C tracers.
(3) Quality control of cigarettes (determination of nicotine, TPM, tar, and ^{14}C delivery of selected radiolabeled cigarettes).
(4) Shipping of radiolabeled cigarettes to Microbiological Associates.
(5) Construction and testing of apparatus for sampling exposure chamber.
(6) Instruction and assistance on operation of smoking machines, sampling apparatus, and cigarette conditioning and handling.
(7) Analysis of animal tissues for ^{14}C tracers.
(8) Analysis of input Cambridge filter pads for nicotine and ^{14}C tracer and chamber samples for ^{14}C tracer.
(9) Calculations to obtain the following results:
    (a) absolute activity (dpm) of tracer in each tissue specimen.

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Source: https://www.industrydocuments.ucsf.edu/docs/hmpf0227

Responsibilities of ORNL (continued)

(9) (b) absolute tar (mg) deposited in each tissue specimen based on tracer deposition.
(c) percent distribution of activity among various tissue specimens for each animal.
(d) dose expressed as percentage of smoke input to exposure chamber.

Responsibilities of Microbiological Associates are the following:

(1) Calibration of smoking machines (puff volume, puff time, exposure time, purge time).
(2) Conditioning and weighing of labeled cigarettes. After receipt from Oak Ridge National Laboratory.
(3) Collection of samples of smoke input to smoking machines and chamber samples obtained during animal exposures.
(4) Animal conditioning.
(5) Animal exposure.
(6) Animal sacrifice and dissection.
(7) Shipping of tissue specimens, input filter pads, and chamber samples to Oak Ridge National Laboratory.

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Experiment I. Effect of Exposure Time

One mouse strain: C3H/fMai

One sex: female

Four exposure time intervals: 10, 20, 30, 40 seconds

One exposure concentration: 10%

Four tissue samples: skinned head, upper trachea and larynx, lungs and lower half of trachea, stomach and esophagus

Number of mice per exposure: 10 (plus 10 scrubs)

Number of cigarettes per exposure: 3 (2 for chamber input, 1 for exposure)

Chamber samples per exposure: 1

Cigarette type: Kentucky Reference 1A1 loaded with 5×10^6 dpm ^{14}C-dotriacontane--weight and RTD tested ($\pm$ 20 mg, $\pm$ 5 mm H_2O)

Number of repetitive exposures: 4 (40 mice)

Instant sacrifice

Tissue specimens: 640

Total Cigarettes: 60 (assume 25% loss).

Experiment II. Effect of Smoke Concentration

One exposure time interval: based on experiment I

Five exposure concentrations: 20% (2 cigarettes--384 ml chamber),
30% (3 cigarettes--384 ml chamber),
5% (1 cigarette--768 ml chamber),
10% (2 cigarettes--768 ml chamber),
15% (3 cigarettes--768 ml chamber)

Number of cigarettes per exposure: 6 (20%), 9 (30%), 3 (5%), 6 (10%), 9 (15%)

Other conditions: same as experiment I

Total tissue specimens: 800

Total cigarettes: 165 (assume 25% loss).

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Source: https://www.industrydocuments.ucsf.edu/docs/jpyf0000

Experiment III. Effect of Sex

Two sex: male and female

One exposure time interval: based on experiment I

One exposure concentration: based on experiment II

Number of mice per exposure: 20 (10 male, 10 female)

Other conditions: same as experiment I

Tissue specimens: 300

Total cigarettes: 15 (assume 25% loss).

Experiment IV. Strain Differences

Four mouse strains: C3H/f, C57BL/6, DBA/2, BC3F1

Sex: based on experiment III (male, female, or both)

One exposure time: based on experiment I

One exposure concentration: based on experiment II

Four tissue samples: experiment I

Number of mice per exposure: 20 (10 each of 2 strains or sexes)

Other conditions: same as experiment I

Tissue specimens: 640 (1 sex)
1280 (2 sexes)

Number of cigarettes: 30 (1 sex)
60 (2 sexes) (assume 25% loss).

Experiment V. Retention Period of ^{14}C-Dotriacontane

One mouse strain: C3H/f

One sex: based on experiments III and IV

One exposure time: based on experiment I

One exposure concentration: based on experiment II

Five tissue samples per animal: same as experiment I plus composite sample of all other animal tissues--animal skinned--skin not included in composite.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjmf0000

Experiment V (continued)

Number of mice per exposure: 20

Five sacrifice times after smoking: 0.25, 1, 4, 16, 24 hours. Four animals sacrificed at each time.

Number of cigarettes per exposure: 3

Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C-dotriacontane.

Number of repetitive exposures: 4

Tissue specimens: 400

Number of cigarettes: 15 (assume 25% loss).

Experiment VI. Retention Period of ^{14}C-Benz(a)pyrene

Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C-benz(a)pyrene

Other conditions: same as experiment V

Tissue specimens: 400

Number of cigarettes: 15 (assume 25% loss).

Experiment VII. Retention Period of ^{14}C-Nicotine

Five sacrifice times after smoking: immediately, 0.25, 0.5, 1, 4 hours after smoking. Four animals sacrificed at each time.

Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8) ^{14}C-nicotine

Other conditions: same as experiment V.

Tissue specimens: 400

Number of cigarettes: 15 (assume 25% loss).

Experiment VIII. Comparative Retention Periods for Two Mouse Strains

Two strains: based on experiment IV

One sex: based on experiments III and IV

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Source: https://www.industrydocuments.ucsf.edu/docs/phyl0000

Experiment VIII (continued)

One exposure time: based on experiment I

One exposure concentration: based on experiment II

Two retention periods: based on experiments V, VI, and VII

Five tissue specimens per animal: experiment V

Twenty mice per exposure: ten mice sacrificed at each retention period

Number of repetitive exposures: 2

Three type cigarettes: Kentucky reference 1A1 loaded with ^{14}C-nicotine, ^{14}C-benz(a)pyrene, or ^{14}C-dotriacontane (maximum load)

Tissue specimens: 1200

Number of cigarettes: 15 (assume 25% loss).

Experiment IX. Cumulative Dose and Long Term Retention

Number of radiolabeled cigarettes given per 8 hour day: 2, 5, 10, 20

One strain - based on experiment VIII

Four animals sacrificed at each of the following times after exposure: immediately, 4, 24, 48, 120 hours

Three type cigarettes: same as experiment VIII

Number of repetitive tests: 1

Other conditions: same as experiment VIII

Tissue specimens: 1200

Number of cigarettes 169 (assume 25% loss).

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https://www.industrydocuments.ucsf.edu/docs/xfbl0000

Summary of Tissue Specimens and Number of Cigarettes

Experiment	Tissue Specimens[1]	Number of Cigarettes[2]
I	640	60
II	800	165
III	320	15
IV	1280	60
V	400	15
VI	400	15
VII	400	15
VIII	1200	15
IX	1200	169
	6640	529

[1] Maximum

[2] Assume 25% loss

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Source: https://www.industrydocuments.ucsf.edu/docs/pbkb0000

ANIMAL CARCINOGENESIS
MODEL

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Source: https://www.industrydocuments.ucsf.edu/docs/jmyl0000

WHITMIRE - M.A.

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